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Trypanosoma evansi in Indonesian buffaloes: evaluation of simple models of natural immunity to infection

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SUMMARY

Deterministic models were employed to investigate the biology of *Trypanosoma evansi* infection in the Indonesian buffalo. Models were fitted to two age-structured data sets of infection. The Susceptible–Infected–Susceptible (SIS) model was the best supported description of this infection, although the results of the analysis depended on the serological test used: the Tr7 Ag-ELISA was judged the most reliable indicator of infection. Estimated forces of infection increase with age from 1·2 to 2·0 acquisitions per buffalo per year. The buffaloes would clear infection in an estimated mean time period of 16·8 months (95% CIs: 12·5–25·9 months) since acquisition, either by drug treatment by owners or self-cure. A general discussion on the role of immunity in protozoan infections includes consideration that the fitted SIS model would be consistent with strain-specific immunity. The model may become a useful tool for the evaluation of control programmes.

INTRODUCTION

Trypanosomosis ('surra') caused by *Trypanosoma evansi* is considered to be one of the most important diseases of horses, cattle and buffaloes in Indonesia. *Trypanosoma evansi* is a blood-borne parasite that is transmitted mechanically by the bites of haematophagous flies. Initially, trypanosomes may be seen readily in the bloodstream, but in chronic infections parasites are difficult or impossible to find [1]. In Indonesia, *T. evansi* typically causes a chronic infection in which weight loss and anaemia are the most characteristic signs of disease. Other clinical signs that may be exhibited are fever, diarrhoea, oedema, jaundice, conjunctivitis, swelling of lymph nodes, abortion and infertility, incoordination and paralysis. Pathological lesions of myocarditis, necrosis of spleen and liver and interstitial pneumonia have been described also [2].

Infection is widespread throughout the archipelago and is present on all the main islands, including Irian Jaya [3]. Occasional epidemic outbreaks of disease occur, but in Indonesia production losses caused by *T. evansi* are an endemic problem (Luckins, unpublished data). These include reduced draught power, due to chronic forms of the disease. In recent years there has been widespread movement of livestock throughout the islands and this must have facilitated the spread of infection, although to what extent is not clear. The data upon which the models have been derived were obtained from surveys of swamp buffaloes carried out in five districts of Central Java, namely, Pemalang, Tegal, Pekalongan, Brebes and Batang [4]. The animals belonged to smallholder and landless farmers who used them for draught purposes for preparation of rice paddies or sugar-cane harvesting. Although individual farmers own only 1–4 buffaloes, the animals

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from the village are kept in communal Housing (kandangs) comprising up to 100 animals. Under these conditions the transmission of infection by the biting fly vectors of *T. evansi* is thereby facilitated. In this paper we present an attempt to evaluate the force of infection in animals born or bought into such groups. Parasitic infections can be treated in a general framework for micro-parasite infections where the unit of analysis is the state of the host, i.e. susceptible, infected, immune [5]. Here we attempt to model the observed age dependent pattern of all *T. evansi* infection among non-diseased buffaloes.

MATERIALS AND METHODS

The data

Two age-stratified data sets of *T. evansi* infection were used to select the most probable models (Table 1). For the estimation of forces of infection and rates of recovery from infection we obtained estimates from two age-stratified data sets of infection, each collected from the same population of infected buffaloes, but differing in the diagnostic test used. One data set was based on the 2G6 Ag-ELISA, and the other was based on the Tr7 Ag-ELISA. The two ELISAs are tests for detection of *T. evansi* infection, with known point estimates of diagnostic sensitivity and specificity. The 2G6 Ag-ELISA has a 71% sensitivity and 75% specificity, and the Tr7 Ag-ELISA has 81% sensitivity and 78% specificity [4]. The following expression was used to correct the raw data to estimate 'true prevalence', P, from the observed 'test prevalence', P^{T}, test sensitivity (s_1) and specificity (s_2) [6]:

$$P = \frac{P^{\mathrm{T}} + s_2 - 1}{s_1 + s_2 - 1}.$$

The mathematical models use age specific prevalence of infection, $P^M - (a)$, as estimates of the true prevalence P for a given age class. The same principles illustrated here were therefore used for fitting the model to the data but in the reverse order, where P^M was translated to a prevalence $P^{M'}$ comparable to the test prevalence, P^{T} (raw data in Table 1). $P^{M'}$ is given by,

$$P^{M'} = P^M . s_1 + (1 - P^M) . (1 - s_2).$$

A general mathematical model for *T. evansi*

In developing a model of the dynamics of *T. evansi*, age-structured deterministic models were compared to observed epidemiological pattern. The general model is illustrated schematically by a flow diagram in Figure 1, where boxes represent categories of infection and the arrows represent the movements between them. We assume that buffaloes are born into a susceptible class. The model only considers asymptomatic infection, which is acquired at a rate known as force of infection, λ. Within the susceptible–infected–resistant (immune)–susceptible (SIRS) model framework infection persists for a mean time $1/r$ after which the recovered buffaloes are immune for a mean time $1/b$, after which they become susceptible again. The model is represented by the set of differential equations,

$$\frac{\mathrm{dS}}{\mathrm{d}a} = \mathrm{R} . b(a) - \mathrm{S} \cdot \lambda(a),$$

$$\frac{\mathrm{dI}}{\mathrm{d}a} = \mathrm{S} . \lambda(a) - \mathrm{I} . r(a),$$

$$\frac{\mathrm{dR}}{\mathrm{d}a} = \mathrm{I} . r(a) - \mathrm{R} . b(a),$$

S, I and R represent the proportion of susceptible, infected and resistant buffaloes such that $\mathrm{S} + \mathrm{I} + \mathrm{R} = 1$. Boundary (starting) conditions are: $a = 0$, $\mathrm{S}(0) = 1$, $\mathrm{I}(0) = 0$, $\mathrm{R}(0) = 0$. This framework includes the SIRS models ($r > 0$, $1/b > 0$), the SIR models ($r > 0$, $1/b = 0$), the SIS models ($r > 0$, $b \to \infty$), and the SI models ($r = 0$). The analysis was repeated for each of the SI, SIS, SIR, and SIRS model forms. The force of infection, $\lambda(a)$, the rate of recovery from infection, $r(a)$ and the rate of loss of immunity, $b(a)$ may be age dependent. Hosts were assumed to live up to age 14 years [4].

Sensitivity analysis and model fitting techniques

The goodness of fit of the models was given by minimizing the binomial deviance, L, between model and data likelihoods. The deviance is asymptotically chi-square-distributed with $M - p$ degrees of freedom, where M is the number of observations (age classes) and p is the number of fitted parameters [7]. We identified models that minimized the binomial log-likelihood deviance, given by the expression:

$$L = 2 . \sum_{a=1}^{a=M} \left(N_{\mathrm{I},j} . \ln \left(\frac{N_{\mathrm{I},j}}{P_j^{M'} . N_{\mathrm{T},j}} \right) + (N_{\mathrm{T},j} - N_{\mathrm{I},j}) . \ln \left(\frac{(N_{\mathrm{T},j} - N_{\mathrm{I},j})}{N_{\mathrm{T},j} \cdot (1 - P_j^{M'})} \right) \right).$$

The data set is prevalence of infection ($N_{\mathrm{I},j}/N_{\mathrm{T},j}$), where $N_{\mathrm{I},j}$ is the number of infected buffaloes in age class j, ($j = 1$ to M) and $N_{\mathrm{T},j}$ is the total number of

Table 1. *The age-stratified data sets of T. evansi infection in Indonesian buffalo [4] that were used in the estimation of forces of infection. In the analyses the midpoints were used for each age class when comparing observations with model predictions.*

	2G6 ELISA		Tr7 ELISA	
Age group (months)	Number of buffalos tested	Number of positive buffalos	Number of buffalos tested	Number of positive buffalos
0–6	48	17	31	14
7–12	207	91	146	73
13–24	390	177	307	195
25–36	403	184	330	205
37–60	548	293	434	295
61–84	442	235	364	239
> 84	401	226	268	172

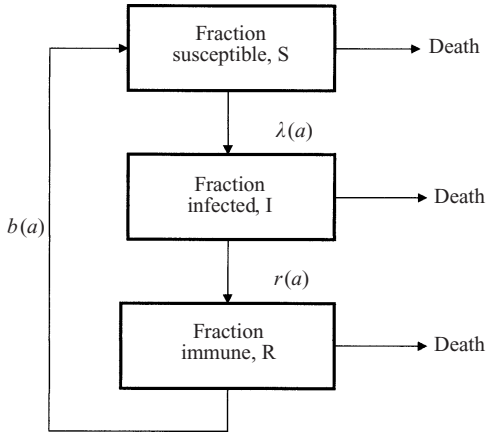

Fig. 1. A schematic representation of the model of *T. evansi* infection in the buffalo. All buffalo are assumed to be born in the susceptible class and then acquire infection at an age-specific rate $\lambda(a)$. Further details are in the text.

buffaloes sampled within age class j. $P_j^{M'}$ is the expected prevalence generated by the model in age class j. A satisfactory model was defined as one where the deviance was not significant ($P > 0{\cdot}05$). Thus a high probability (P-value) indicates that the deviance between data ($N_{I,j}/N_{T,j}$) and model ($P_j^{M'}$) may have arisen by chance. A model with a smaller deviance, L, is said to have greater support [8].

The 95% confidence intervals for the estimates of model parameters were estimated as the range of all parameter combinations which correspond to a deviance, L, within $\chi^2_{\nu,\alpha}$ of the minimum L where ν (the number of degrees of freedom) is the number of fitted parameters and $\alpha = 0{\cdot}05$ [9].

Assumptions of the models

The models used in these analyses are simple and ignore a number of complexities such as parasite or host heterogeneities and the population dynamics of vectors. It was also assumed that in Java *T. evansi* behaves as an endemic infection, which may be reasonable, given the endemic pattern of surra disease it causes. It is considered as a first necessary step in modelling these parasites.

The models of *T. evansi* infection did not include details of the demography of the buffalo population apart from the assumption that all animals die at age 14 years. This is not a problem as far as the objectives of this paper are concerned, which are to estimate rates of recovery per infection and rates of acquisition of infection per susceptible buffalo. This means that the analyses depend on the *proportion* infected at each age class and the demography is irrelevant. However, detailed information on the demography of the buffalo group would be desirable when using the models to simulate a control programme, as, in this case it is the *number* of cases of infection and disease that matters.

RESULTS

The SI and the SIS models both fitted the 2G6 Ag-ELISA and Tr7 Ag-ELISA data sets adequately. The SIRS model form also fitted the data, but it was not distinguishable from the fitted SIS model as its estimated duration of immunity was effectively equivalent to an SIS model. The assumption of an age-dependent force of infection, $\lambda(a)$, and a constant recovery rate from infection, r, received the strongest support from the data sets. The functional form of the force of infection that received greatest support was,

$$\lambda(a) = \alpha(a-\gamma)\exp(-a/\beta)+\gamma.$$

Where α represents the rate of increase of $\lambda(a)$ at birth, β represents the age for the peak of λ(a), and γ

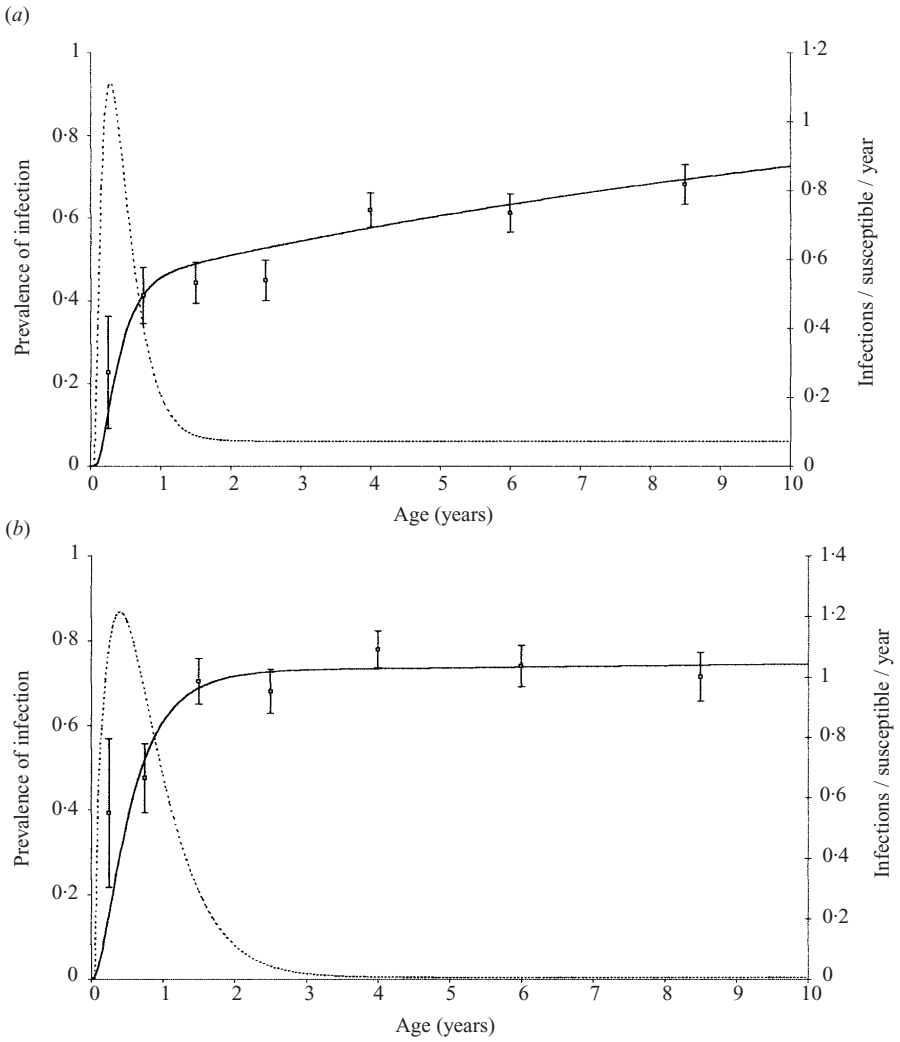

Fig. 2. SI model fits to the raw data ($P^{M'}$ in the text, with 95% error bars) and estimated age-dependent forces of infection $\lambda(a)$ (dotted line). (*a*) Parameter estimates from the 2G6 ELISA data set were $\alpha = 20{\cdot}08$ (13·20, 26·44); $\beta = 0{\cdot}195$ (0·154, 0·257); $\gamma = 0{\cdot}0821$ (0·0572, 0·1067), and $R_0 = 2{\cdot}68$. (*b*) For the Tr7 ELISA data set these were $\alpha = 7{\cdot}88$ (5·99, 9·58); $\beta = 0{\cdot}411$ (0·345, 0·513); $\gamma = 9{\cdot}27 \times 10^{-5}$ ($7{\cdot}29 \times 10^{-5}$, $11{\cdot}38 \times 10^{-5}$).

represents the asymptotic value of λ(a) after the peak. This functional form is quite flexible, and does not necessarily constrain functional forms to having peaks [10, 11]. Other model forms, especially those with age dependent rates of recovery from infection, $r(a)$, failed to provide better fits. We will restrict ourselves to the models that were best supported by the data sets.

The SI models

SI model forms that received the best support from the data assumed a peak in the force of infection, λ(a) in the first year of age. After this age the force of infection declines to zero (Fig. 2*a*, *b*). The 2G6 Ag-ELISA gave a deviance of 4·03 ($P = 0{\cdot}26$), the Tr7 Ag-ELISA gave a deviance of 6·0 ($P = 0{\cdot}11$).

The SIS models

The greatest degree of support was obtained with the SIS model form, where infected buffaloes recover from infection (by treatment or self cure) but remain susceptible to re-infection with *T. evansi*. Both data sets estimated a force of infection that increases with age, without reaching a peak (Fig. 3*a*, *b*). The 2G6 Ag-ELISA test a deviance of 2·48 ($P = 0{\cdot}65$) and the Tr7 Ag-ELISA gave a deviance of 4·046 ($P = 0{\cdot}39$).

DISCUSSION

Age-stratified data of *T. evansi* infection were compared to model prediction to provide insights on the nature of the interaction between these protozoan parasites and their hosts. Assuming that *T. evansi* is at

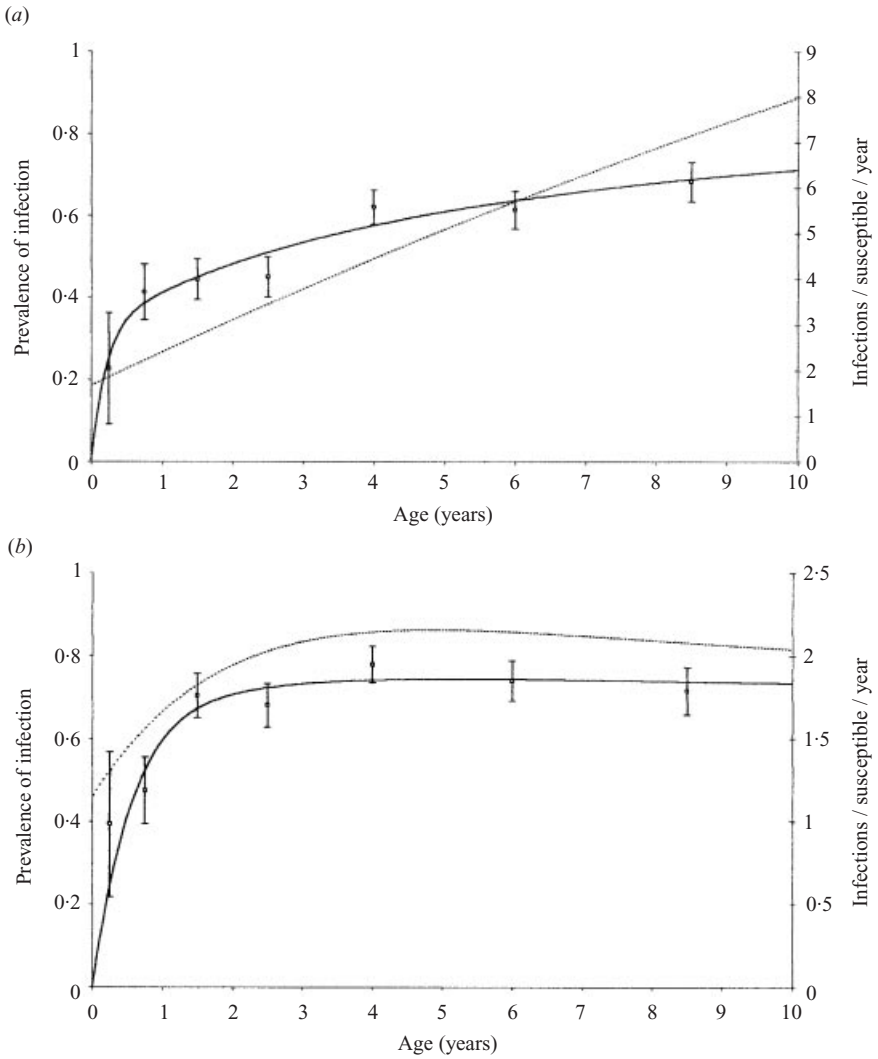

Fig. 3. SIS model fits to the raw data ($P^{M\prime}$ in the text, with 95% error bars) and estimated age-dependent forces of infection $\lambda(a)$ (dotted line). (*a*) The parameter estimates (95% confidence intervals in brackets) from the 2G6 ELISA data set were $\alpha = 0{\cdot}711$ (0·502, 0·923); $\beta = 49{\cdot}79$ (35·10, 64·65); $\gamma = 6{\cdot}004$ (4·212, 7·801); $r = 3{\cdot}466$ (2·433, 4·491) and $R_0 = 2{\cdot}61$. (*b*) The parameter estimates for the Tr7 ELISA data set were $\alpha = 0{\cdot}379$ (0·250, 0·511); $\beta = 2{\cdot}984$ (1·950, 4·021); $\gamma = 1{\cdot}852$ (1·215, 2·472); $r = 0{\cdot}714$ (0·464, 0·959).

endemic equilibrium, the models that most successfully fitted the data were those suggesting either the inability of hosts to recover from infection (SI models), or, if there is recovery, the models suggest that hosts remain susceptible to re-acquisition of infection (SIS models). The two sets of models did not differ in their ability to fit the data sets. The difference between these models is that the SIS model does not restrict the force of infection to the first year of life (cf. Fig. 2 with Fig. 3). Other models, such as SIR models and models that assume age dependent recovery rates, $r(a)$, were poorly supported by the data, and SIRS model did not fit the data better than the SIS model.

The SI models require the assumption that requisition of infection is restricted to buffaloes in the first year of life. This is thought unlikely in the light of the fact that Tabanid vectors bite (and transmit infection to) all age classes. Nevertheless, the fitted SIS models do imply an age-dependent force of infection where acquisition of infection rises with age, especially during the first 2 years of life of the buffalo host. This may be consistent with the effect of buffalo body size. Host size and shape has been linked to vector biting activity for *Anopheles gambiae* mosquitoes and the tsetse fly [12, 13].

Depending on the data set fitted, the SI models suggest that forces of infection peak at values of 1·05 (2G6 Ag-ELISA) and 1·20 (Tr7 Ag-ELISA) yearly acquisitions per buffalo. For the SIS model, the 2G6 Ag-ELISA data set predicts greater forces of infection

that range from 1·85 to 7·16 acquisitions per buffalo per year, and the Tr7 Ag-ELISA data set predicts between 1·31 and 2·08 yearly acquisitions per buffalo. Furthermore, the SIS models suggest estimated mean duration of infection between 3·5 months (95% CI: 2·7–4·9 months) (2G6 Ag-ELISA), and 16·8 months (95% CI: 12·5–25·9 months) (Tr7 Ag-ELISA). An explanation may be that the Tr7 Ag-ELISA detect antigens that are expressed earlier in the course of infection than those detected with 2G6 Ag-ELISA. This is supported by the fact that the Tr7 Ag-ELISA detect more infection in the younger age classes, and may be the more accurate indication of infectious status throughout age groups (cf. Fig. 3*a* and *b*). An independent longitudinal study where buffaloes were followed up for detection *T. evansi* infection in the Kebumen and Gejlig villages (A1 and B1 in ref. [4]) estimates an exact incidence rate (force of infection) of 0·44 per buffalo per year using the Tr7 Ag-ELISA test (95% CI: 0·24, 0·76) [4]. This estimate is closer to that estimated by the Tr7 Ag-ELISA, although the significance of this result must be taken with caution because this direct estimate is likely to be an underestimate, as it is based on the assumption that the force of infection is age-independent with no recovery rate. The observation that different epidemiological parameter estimates are obtained by using data based on different, but well-characterized, diagnostic tests is relevant to many other studies.

Our interpretation of the cross-sectional data of *T. evansi* infection of buffaloes suggests recovery from infection. This may be through treatment with trypanocidal drugs or by self-cure (although treatment is only carried out by government vets, and only if *clinical* trypanosomosis occurs). The latter alternative is possible although the frequency of its occurrence is not known. In the field, Randall and Schwartz [14] identified a number of cattle that appeared to have recovered from infection with *T. evansi* and several workers have suggested that buffaloes also show spontaneous recovery from infection [15–17]. Onah et al. [18] were able to demonstrate self-cure in experimentally infected sheep; animals that eliminated trypanosome infection were shown to have qualitative and quantitative differences in the production of different classes of lymphocytes compared with animals that were unable to clear the infection. What the immune status of such animals might be is not clear.

The data would suggest that if buffaloes do clear infection they remain susceptible to its re-acquisition at a later time (SIS model). These are possibilities that have long been considered for protozoan infections. In a review of all known parasite infections, Sergent [19] proposed a general classification scheme for the characteristics of the host–parasite interaction that included a range of 'epidemiological behaviours' consistent with SI and SIS models. Among the protozoan parasites Sergent used the example of animal malaria and *Theileria* organisms, with very long latent infection, approximating the SI models. Because such infections are disease-free Sergent called this form of 'relative immunity' *premunition*. The theory of premunition suggests that although the immune system of the host is incapable of resisting infection, it will act to prevent superinfection by a parasite, and therefore protects against disease associated with superinfection.

Furthermore, according to Sergent, many malarias, piroplasmoses, trypanosomoses, and coccidioses belong to the pattern represented here by the SIS models. Part of the failure of the immune system to prevent re-acquisition of infection may be explained by evolution of immune evasion mechanisms on behalf of the parasite. In their review, Roberts and Janovy [20] mention the example of pathogenic trypanosome infections where a 'variant-specific surface glycoprotein (VSG)' acts as a 'moving target' for the host immune system [21]. SIS models would be consistent with this picture wherever immunity to infection is variant specific. Although the SIS models investigated here do not explicitly consider strain structure in the parasite population, they are consistent with the scenario where a host acquires immunity to a strain of *T. evansi*, and clears it. If the number of variants is large, it would be unlikely that the host acquire *T. evansi* of the same variant on re-infection, and its immune system would be unable to combat this next infection. This is essentially an SIR model (susceptible-infected-resistant) model with variant-structure imposed upon it, such that the host population would be structured by the proportion resistant to a combination of the *n T. evansi* strains locally present. This theory depends on 1) the diversity of strain structure of the *T. evansi* population, and 2) on the strain specific nature of the protective immune response. The duration of freedom from infection would depend on the diversity of the trypanosome population: immune animals recovered from one infection would remain susceptible to infection with parasites presenting different antigenic repertories. Attempts to model such host–parasite interactions

exist but their complexity has restricted the investigations to populations with only up to three parasite variants [22], and only two host genotypes where frequency dependent selection was considered [23]. The usefulness of these studies has been limited by the lack of biological data needed to estimate the considerable number of parameters that are involved. There has been little attempt to determine population diversity in *T. evansi* although there is experimental evidence of variant-specific immunity [24]. Studies on the molecular characteristics of isolates of *T. evansi* from buffaloes in a number of villages in Central Java have shown variation in molecular karyotypes during an 18-month period of observation (unpublished observations). However, this diversity has not been related to strain specificity and the possibility that this could influence the nature of the immune response. In other studies, polymorphisms in DNA has been observed in relation to the electrophoretic karyotypes of different isolates. The presence of a particular karyotype was related to the application of different trypanocidal drugs [25].

The vector population was not explicitly considered. This does not invalidate the analyses as infection in the vector population is directly affected by infection in the host, without significant time lags, and therefore the role of vectors can be ignored and their effects implicitly assumed within the estimated forces of infection $\lambda(a)$ [26]. Transmission of *T. evansi* occurs via different species of haematophagous biting flies, including *Tabanus, Stomaxys, Haematopota, Chrysops* and *Lyperosia* spp. These flies are aggressive feeders, and their vigorous attacks on the host cause defensive reactions that disturb the flies so that they fly to other hosts, in order to complete their blood meal. This form of interrupted feeding enables transmission of the trypanosomes; flies feeding on an infected animal initially complete feeding on an uninfected host. Successful transmission depends on the survival of the trypanosomes present in blood trapped in the fly's mouthparts and even a single fly can transmit infection on several occasions [27]. Survival periods of trypanosomes on the flies' mouthparts varied from as short as a few minutes to as long as 3 days, but it is generally agreed that transmission succeeds if feeding takes place within 1 h of the infective feed. The probability of transmission is 0·05 within 5 min of an infective feed, decreasing to 0·04 by 60 min, 0·001 within 3 h and 0·0003 at 24 h [28]. Clearly, duration of infection is so short that prevalence is directly dependent on that in the hosts.

The link between infection and disease has not been investigated in this work although it would be desirable to devise a model with a diseased class for the purposes of simulating control programmes. There is evidence that apparently uninfected (aparasitaemic) animals, showing no clinical signs can develop acute disease when subjected to stress including work, inclement weather or other, intercurrent, infections [29]. Further investigations are needed to obtain a clearer understanding of the course of infection with *T. evansi* in naturally infected ruminants in relation to its persistence, elimination and the likely consequences this could have on its transmission of *T. evansi*.

In conclusion we have illustrated how simple mathematical models of the spread of *T. evansi* infection within buffalo groups can improve our interpretation of available data. These models could be developed as tools to investigate the feasibility and cost-effectiveness of control programmes. This kind of investigation however requires good quality information on the demography of the buffalo groups and an improved understanding of the incidence of disease ('surra') and immunity to disease.

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